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First Report of Two Cephalobidae Species (Nematoda: Cephalobomorpha) in South Korea

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ABSTRACT

Cephalobus aff. *quinilineatus* (Shavrov, 1968) Anderson and Hooper, 1970 and *Eucephalobus hooperi* Marinari-Palmisano, 1967 from the family Cephalobidae Filipjev, 1934 (Cephalobomorpha) are newly reported from South Korea. *Cephalobus* aff. *quinilineatus* is distinguished from other *Cephalobus* species by its high and rounded labial probolae and five lateral incisures, with three incisures extending to the tail terminus. *Eucephalobus hooperi* is distinguished from other *Eucephalobus* species by its three bifurcated labial probolae with pointed termini and by morphometric characters such as body and tail length and the corpus:isthmus ratio. In this study, the morphological characters and morphometrics of *C*. aff. *quinilineatus* and *E. hooperi* Korean population are described and illustrated based on optical and/or scanning electron microscopy.

Keywords: Cephalobidae, *Cephalobus*, *Eucephalobus*, new record, South Korea

INTRODUCTION

Members of the family Cephalobidae Filipjev, 1934 are bacterial feeders and are widely distributed in almost all terrestrial environments, including the dry soils of Antarctica and desert environments and tropical rainforests (Loof, 1971; Timm, 1971; Waceke et al., 2005; Nadler et al., 2006; Amirzadi et al., 2013). They are morphologically and taxonomically one of most problematic free-living nematode families (Andrássy, 2005) due to morphological and morphometric variation (such as body size, cephalic and labial probolae shape, nerve ring and excretory position and tail form) (Anderson, 1968; Anderson and Hooper, 1970, 1971; De Ley et al., 1993). The variability and similarity of morphological characters among Cephalobidae species obstruct species identification and phylogenetic studies for this group (Rashid et al., 1988; Boström, 1993; Abolafia and Peña-Santiago, 2002, 2005, 2009). Currently, 38 genera have been established in this family; however, only 29 can be accepted as valid (Andrássy, 2005). In Korea, 5 species have been reported (Eun et al., 2016; Kim et al., 2016; Kim et al., 2017a, 2017b, 2017c): *Acrobeles ciliatus* von Linstow, 1877; *Acrobeloides nanus* (de Man, 1880) Anderson, 1968; *A. varius* Kim, Kim and Park, 2017; *Eucephalobus oxyuroides* (de Man, 1876) Steiner, 1936; and *Pseudacrobeles* (*Pseudacrobeles*) *curvatus* Kim, Kim and Park, 2017.

During a survey of several overgrown fields, natural forest and plots of farmland, *C*. aff. *quinilineatus* (Shavrov, 1968) Anderson and Hooper, 1970 and *E. hooperi* Marinari-Palmisano, 1967 were collected and isolated from soil samples. Here we provide detailed descriptions of the morphological characters and morphometrics of the Korean isolates of *C*. aff. *quinilineatus* and *E. hooperi*.

MATERIALS AND METHODS

Nematode isolation

Soil samples were collected from the ground below pear trees (Gongdo-eup, Anseong-si, Gyeonggi-do, South Korea [GPS coordinates: 37°01′01.1″N, 127°10′23.4″E]), overgrown fields (Mechuri Island, Daenam-ro, Danwon-gu, Ansan-si, Gyeonggi-do, South Korea [GPS coordinates: 37°12′02.9″N, 126°32′24.6″E]), and natural forest areas

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(Geojejungang-ro, Dongbu-myeon, Geoje-si, Gyeongsangnam-do, South Korea [GPS coordinates: 34°47′04.2″N, 128°37′30.3″E]). Nematode specimens were extracted by sieving and the Baermann funnel method (Baermann, 1917).

Fixation and morphological observations

For fixation, nematode specimens were transferred to 2 mL purified water in a 15 mL tube, to which was quickly added 4 mL of 80°C TAF (2% triethanolamine and 7% formaldehyde). The fixed nematodes were dehydrated in accordance with Seinhorst (1959) and mounted in pure glycerin on HS slides (Shirayama et al., 1993). Nematode morphological characters were observed under an optical microscope (BX-51; Olympus, Tokyo, Japan) equipped with differential interference contrast. Morphometric characters were measured using a CoolSnap Photometrics color CCD digital camera (MP5.0-RTV-R-CLR-10; Photometrics, Tucson, AZ, USA) and the program QCapture Pro 5 (QImaging, Surrey, Canada).

Scanning electron microscope (SEM)

For SEM imaging of *C*. aff. *quinilineatus*, the fixed specimens were transferred to an aqueous osmium tetroxide solution (4%) and kept at 4°C for 1 day for post-fixation. Fixed nematode specimens were dehydrated through a series of increasing ethanol concentrations (10–100% absolute ethanol, with 1 hour per stage). The samples were dried using a Hitachi HCP-2 critical point drier (Tokyo, Japan). Dried nematodes were mounted on a stub using copper/nickel tape and coated with gold/palladium using an Eiko IB-3 sputter-coater (Tokyo, Japan). Morphological characters of the nematode specimens were observed with a Zeiss Ultra Plus SEM (Oberkochen, Germany) at 15 kV under high-vacuum conditions.

SYSTEMATIC ACCOUNTS

Order Rhabditida Chitwood, 1993
Suborder Tylenchina Thorne, 1949
Infraorder Tylenchomorpha De Ley and Blaxter, 2002
Family Cephalobidae Filipjev, 1934
[1*]Genus *Cephalobus* Bastian, 1865

[2*]*Cephalobus* aff. *quinilineatus* (Shavrov, 1968) Anderson and Hooper, 1970 (Table 1, Figs. 1, 2)

Chiloplacus quinilineatus: Shavrov, 1968: 137, fig. 1.
Acrobeloides amurensis: Truskova, 1971: 434, fig. 1.
Cephalobus quinilineatus: Anderson and Hooper, 1970: 468.

Material examined. 5♀♀ and 3♂♂, South Korea: Gyeonggi-do, Anseong-si, Gongdo-eup, Deokbongseowon-ro, 1 Jan 2017, extracted by sieving and the Baermann funnel method from soil below pear trees. Two specimens (slide Nos. NIBRIV0000812916 [female] and NIBRIV0000812917 [male]) are deposited at the National Institute of Biological Resources, Republic of Korea and six specimens (slide Nos. 01010202002-01010202005 [females] and 01010202007 and 01010202008 [males]) are deposited in the Animal Phylogenomics Laboratory, Ewha Womans University, Republic of Korea.

Measurements. See Table 1.

Description. Adult: Female body cylindrical, length 865.8–962.4 μm; ventrally curved after fixation, C-shaped. Male body cylindrical, length 808.1–908.9 μm; posterior region more curved than female, J-shaped. Cuticle annulated; annuli 2.4–3.1 μm wide and 1.0–1.7 μm thick at mid-body. Lateral field occupying 20.0–24.3% of width of body at mid-body in female, 32.3–35.1% of body diameter in male. Lateral incisures varying in number along body length: two incisures at procorpus region, branching off from posterior corpus into five incisures, two incisures fading out at phasmid region, three incisures extending to tail terminus. Head region continuous with neck. Lip region 9.8–11.9 μm in diameter, with triradiate symmetry with 6+4 papillae. Six lips grouped in pairs; deep U-shaped primary axils; shallow V-shaped secondary axils; lacking guarding processes, without tine. Cephalic probolae absent. Three plate-like, high and rounded labial probolae present. Transversal, oval-shaped amphidial apertures present. Stoma cephaloboid, 1.4–1.6 times the lip region diameter in length; cheilorhabdions oval shaped. Pharyngeal corpus cylindrical, 2.9–4.2 times isthmus length. Isthmus narrower than corpus, distinctly demarcated from metacorpus. Basal bulb oval-shaped with well developed valves; 1.1–1.3 times as long as its width. Cardia conoid, surrounded by intestinal tissue. Nerve ring position varying from posterior corpus to anterior isthmus, at 65.7–72.9% of pharynx length. Excretory pore position similar to nerve ring, at 67.8–79.5% of pharynx length. Deirids positioned in lateral field at level of isthmus or isthmus-basal bulb junction, at 75.7–87.1% of total neck length.

Female: Reproductive system monodelphic-prodelphic. Vulva lips slightly protruding or not. Vagina short, length 0.3–0.6 times the body diameter. Post-uterine sac 0.8–2.1 times the body width. Uterus length 2.9–4.0 times the body diameter. Spermatheca inconspicuous, 1.3–2.1 times the body width. Oviduct short. Ovary with or without double flexure posterior to vulva. Rectum length 1.2–1.5 times the

Korean name: [1*]두옆선충속 (신칭), [2*]오선두옆선충 (신칭)

Table 1. Morphometrics of *Cephalobus* aff. *quinilineatus* and *Eucephalobus hooperi*

Characters	*Cephalobus* aff. *quinilineatus*		*Eucephalobus hooperi*	
	♀, n=5	♂, n=3	♀, n=4 (Ansan)	♀, n=1 (Geoje)
L	902.9±44.4 (865.8–968.4)	848.6±53.3 (808.1–908.9)	416.5±48.9 (366.3–483.1)	452.6
a	21.6±1.7 (19.3–24.0)	24.6±0.9 (23.5–25.3)	21.8±2.2 (19.5–23.9)	21.2
b	4.5±0.3 (4.2–4.8)	4.5±0.3 (4.3–4.9)	3.2±0.2 (2.9–3.3)	3.5
c	20.2±0.5 (19.4–20.6)	16.7±0.8 (16.2–17.6)	9.1±1.1 (7.5–10.1)	10.1
c′	1.9±0.3 (1.7–2.3)	1.8±0.1 (1.7–1.9)	4.1±0.2 (3.8–4.4)	3.5
V	66.4±1.9 (64.8–69.4)	–	67.5±6.2 (63.7–76.7)	65.5
G or T	47.0±2.2 (45.3–50.7)	48.0±8.5 (38.3–54.1)	31.5±6.2 (26.7–40.6)	25.7
Body diameter	42.0±2.7 (38.6–44.9)	34.6±3.5 (31.9–38.6)	19.3±3.7 (16.8–24.8)	21.3
Pharynx length	201.6±12.3 (180.7–212.3)	189.0±4.4 (185.8–193.9)	132.8±22.1 (117.0–165.5)	129.2
Tail length	44.7±2.8 (42.3–47.8)	50.7±1.3 (49.3–51.6)	46.9±11.7 (39.9–64.4)	45
Anal body diameter	23.3±1.7 (20.5–24.9)	27.7±1.9 (26.4–29.9)	11.5±2.2 (10.1–14.7)	12.7
Lip region diameter	11.0±0.8 (9.8–11.9)	10.4±0.2 (10.1–10.6)	5.8±0.9 (5.0–6.8)	5.7
Stoma	15.9±0.4 (15.5–16.3)	15.5±0.7 (14.8–16.2)	10.6±1.2 (9.6–12.3)	10.8
Stoma diameter	6.6±0.6 (5.7–7.0)	6.0±0.2 (5.8–6.1)	4.3±0.6 (3.7–5.1)	4
Stoma/lip region diameter	1.5±0.1 (1.4–1.6)	1.5±0.0 (1.5–1.5)	1.8±0.2 (1.6–2.0)	1.9
Stoma/stoma diameter	2.4±0.2 (2.2–2.8)	2.6±0.2 (2.4–2.8)	2.5±0.1 (2.3–2.6)	2.7
Corpus	119.1±7.6 (109.7–130.0)	107.8±6.2 (103.1–114.8)	75.5±17.5 (61.1–101.0)	73.9
Isthmus	32.7±4.2 (26.0–37.1)	34.7±1.4 (33.7–36.3)	25.7±1.8 (24.4–28.2)	24.4
Basal bulb	25.3±3.3 (20.6–28.7)	22.7±0.5 (22.2–23.1)	17.0±2.7 (14.9–20.9)	15.6
Basal bulb diameter	21.5±1.3 (19.6–22.9)	18.8±0.3 (18.7–19.1)	12.7±1.8 (11.4–15.3)	12.5
Basal bulb length/diameter	1.2±0.1 (1.1–1.3)	1.2±0.0 (1.2–1.2)	1.3±0.0 (1.3–1.4)	1.2
Corpus:isthmus ratio	3.7±0.4 (3.2–4.2)	3.1±0.3 (2.9–3.4)	2.9±0.5 (2.4–3.6)	3
Nerve ring to ant. end	138.1±6.3 (131.4–148.1)	134.5±7.0 (127.6–141.5)	83.0±14.7 (69.2–103.3)	83.1
Excretory pore to ant. end	148.0±6.2 (141.3–156.8)	140.2±7.2 (131.9–144.4)	84.9±14.8 (74.7–106.8)	86.7
Deirid to ant. end	166.5±7.9 (156.2–177.1)	156.4±8.4 (146.9–163.0)	94.4±13.3 (83.3–113.7)	97.5
Nerve ring position (% pharynx)	68.6±3.1 (65.7–72.7)	71.1±2.6 (68.2–72.9)	62.5±3.9 (59.1–67.9)	64.3
Excretory pore position (% pharynx)	73.6±4.3 (67.8–79.5)	74.3±5.4 (68.0–77.7)	63.9±0.5 (63.2–64.5)	67.1
Deirid position (% pharynx)	82.7±3.2 (79.0–86.4)	82.9±6.2 (75.7–87.1)	71.3±1.9 (68.7–72.8)	75.5
Vulva from ant. end	599.3±22.6 (572.7–630.1)	–	283.0±59.6 (236.7–370.6)	296.6
Vulva to anus	258.5±23.3 (229.3–283.4)	–	110.4±24.6 (89.9–145.2)	107.4
Vulva to anus/tail length	5.8±0.3 (5.4–6.2)	–	2.4±0.2 (2.3–2.7)	2.4
Reproductive tract length	424.6±39.5 (399.5–491.4)	408.2±80.4 (317.4–470.3)	133.3±42.5 (105.4–196.4)	116.3
Vagina	19.7±4.0 (13.8–24.3)	–	7.3±1.9 (5.7–10.1)	5.6
Post-uterine sac	72.6±20.8 (37.1–88.9)	–	24.0±2.3 (21.4–26.9)	21.7
Uterus	144.2±14.0 (128.3–156.1)	–	43.1±9.6 (37.8–57.6)	45.3
Spermatheca	65.6±16.5 (52.7–93.2)	–	23.8±7.9 (16.1–33.6)	34.6
Oviduct	17.0±7.0 (9.2–26.7)	–	8.0±2.3 (5.6–11.0)	10.1
Ovary	351.3±33.0 (315.9–392.5)	–	105.3±40.1 (82.4–165.3)	90
Vagina/body diameter	0.5±0.1 (0.3–0.6)	–	0.4±0.0 (0.3–0.4)	0.3
Post-uterine sac/body diameter	1.7±0.5 (0.8–2.1)	–	1.3±0.3 (1.0–1.6)	1
Uterus/body diameter	3.5±0.5 (2.9–4.0)	–	2.2±0.1 (2.1–2.3)	2.1
Spermatheca/body diameter	1.6±0.3 (1.3–2.1)	–	1.3±0.5 (0.9–2.0)	1.6
Oviduct/body diameter	0.4±0.2 (0.2–0.7)	–	0.4±0.1 (0.3–0.5)	0.5
Ovary/body diameter	8.4±0.9 (7.5–9.8)	–	5.3±0.9 (4.6–6.7)	4.2
Spicules	–	50.9±3.4 (47.0–53.6)	–	–
Spicules/body width	–	1.5±0.1 (1.4–1.6)	–	–
Gubernaculum	–	25.8±3.8 (22.3–29.8)	–	–

Table 1. Continued

Characters	*Cephalobus* aff. *quinilineatus*		*Eucephalobus hooperi*	
	♀, n=5	♂, n=3	♀, n=4 (Ansan)	♀, n=1 (Geoje)
Gubernaculum/body width	–	0.7±0.0 (0.7–0.8)	–	–
Gubernaculum/spicules	–	0.5±0.1 (0.4–0.6)	–	–
Rectum	30.8±1.8 (29.0–33.8)	–	15.7±3.9 (12.6–21.4)	16.6
Rectum/anal body diameter	1.3±0.1 (1.2–1.5)		1.4±0.1 (1.2–1.5)	1.3
Anus to phasmid	29.9±3.6 (26.4–35.4)	33.4±2.6 (31.6–36.5)	13.5±1.3 (12.8–15.4)	13.2
Phasmid position (% tail)	66.8±6.6 (61.2–74.3)	65.9±4.5 (61.6–70.6)	30.0±6.9 (20.2–36.6)	29.3
Lateral field width	9.4±1.0 (7.7–10.4)	11.8±0.7 (11.2–12.5)	3.4±0.5 (2.8–4.0)	4.5
Lateral field width/body diameter (%)	22.3±1.7 (20.0–24.3)	34.1±1.6 (32.3–35.1)	17.6±1.6 (16.0–19.4)	21.1
Cuticle thickness	1.3±0.3 (1.0–1.6)	1.4±0.3 (1.1–1.7)	1.2±0.1 (1.0–1.2)	1.2
Annuli width	2.9±0.3 (2.6–3.1)	2.7±0.3 (2.4–3.0)	1.5±0.2 (1.2–1.8)	1.6
Mucro	–	–	4.9±5.1 (1.3–8.5)	–

Measurements are in µm and in the form mean±SD (range).

anal body diameter. Tail conoid, with rounded terminus. Phasmids located behind middle of tail, at 61.2–74.3% of tail length.

Male: Genital system monorchic. Testis reflexed ventrad anteriorly. Spicules curved ventrad, 47.0–53.6 µm long; manubrium rounded or conoid; calamus wider than manubrium; without hump or velum; with one longitudinal incisure. Gubernaculum curved ventrad, sigmoid. Two pairs of pre-cloacal subventral papillae present. Post-cloacal genital papillae in five pairs: one subventral and one lateral at midtail; one lateral, one ventral and one subdorsal at tail terminus region. Tail conoid, blunt terminus. Phasmid opening at 61.6–70.6% of tail length.

Distribution. India, Russia, South Korea.

Habitat. Soil sample from a pear farm.

Remarks. Morphological characters and measurements of the specimens described in this study generally agree with original descriptions of *C. quinilineatus* (Shavrov, 1968) Anderson and Hooper, 1970, except for female body length (865.8–968.4 vs. 642–712 µm) and labial probolae shape (rounded vs. bifurcated) (Shavrov, 1968). Although female body length and labial probolae shape differ between the present specimens and the original description of *C. quinilineatus*, this is not sufficient evidence for a different species diagnosis, due to the varying degrees of bifurcation and rounded labial probolae of the synonymized *A. amurensis* (Shavrov, 1968; Truskova, 1971). Additionally, the original description of *C. quinilineatus* did not include the male and omitted many key characters needed for identification to species level, such as descriptions of the lateral field at the tail region, post-uterine sac, spermatheca and ratio of corpus to isthmus length (Shavrov, 1968). Therefore, it is impossible to be certain about the identity of the specimens. Due to close similarity of the specimens with *C. quinilineatus*, we consider the specimens to be *C.* aff. *quinilineatus*. This species is reported for the first time from South Korea.

Genus *Eucephalobus* Steiner, 1936

[1*]***Eucephalobus hooperi*** **Marinari-Palmisano, 1967 (Table 1, Fig. 3)**

Material examined. 4♀♀, South Korea: Gyeonggi-do: Ansan-si, Danwon-gu, Daenam-ro, Mechuri Island, 22 Sep 2016; 1♀, Gyeongsangnam-do: Geoje-si, Dongbu-myeon, Geojejungang-ro, 5 Jul 2017, extracted by sieving and the Baermann funnel method from overgrown field and natural forest soil, respectively. The two specimens (slide Nos. NIBRIV0000812922 and NIBRIV0000812923) are deposited

Korean name: [1*]후퍼유럽두엽선충 (신칭)

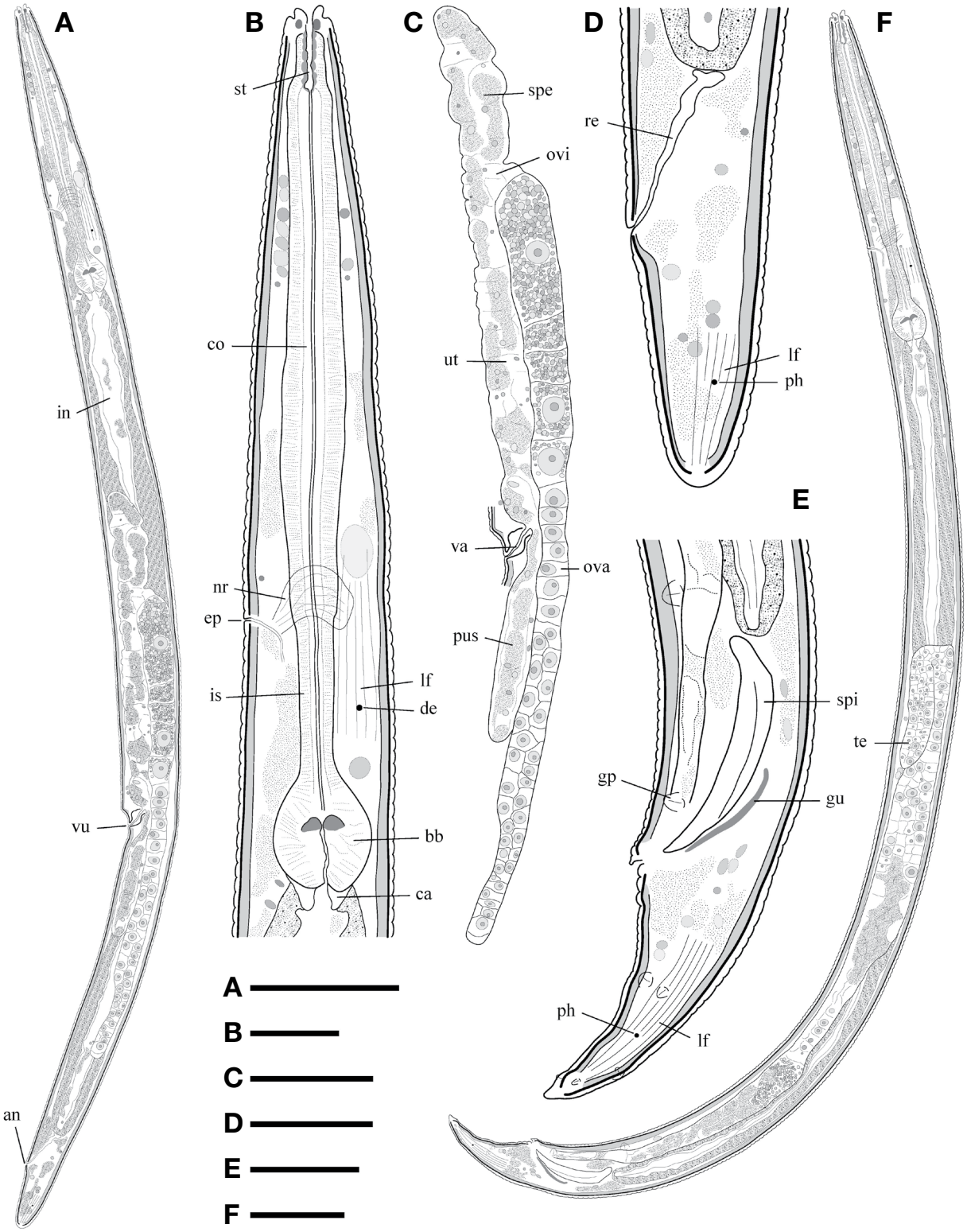

Fig. 1. *Cephalobus* aff. *quinilineatus* (Shavrov, 1968) Anderson and Hooper, 1970. A, Entire female; B, Female neck region; C, Female reproductive system; D, Female posterior region; E, Male posterior region; F, Entire male. an, anus; bb, basal bulb; ca, cardia; co, corpus; de, deirid; ep, excretory pore; gp, genital papilla; gu, gubernaculum; in, intestine; is, isthmus; lf, lateral field; nr, nerve ring; ova, ovary; ovi, oviduct; ph, phasmid; pus, post-uterine sac; re, rectum; spe, spermatheca; spi, spicule; st, stoma; te, testis; ut, uterus; va, vagina; vu, vulva. Scale bars: A = 100 μm, B, D, E = 20 μm, C, F = 50 μm.

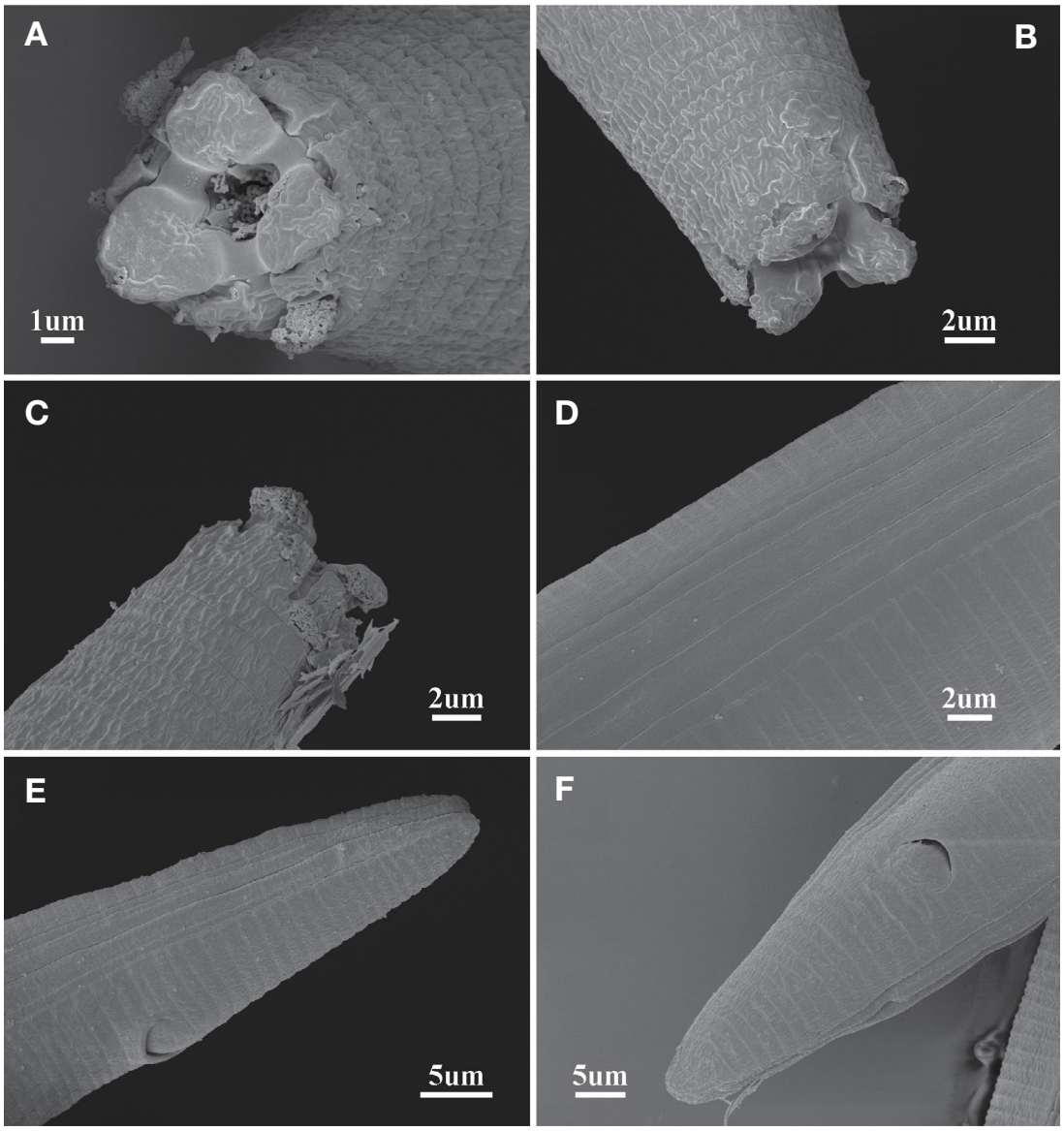

Fig. 2. *Cephalobus* aff. *quinilineatus* (Shavrov, 1968) Anderson and Hooper, 1970. A, Lip region, en face view; B, Lip region, lateral view; C, Lip region, ventral view; D, Lateral field; E, Tail, lateral view; F, Tail, ventral view. Scale bars: A=1 μm, B–D=2 μm, E, F=5 μm.

at the National Institute of Biological Resources, Republic of Korea, and three specimens (slide Nos. 01010103001, 01010103002 and 01010103007) are deposited in the Animal Phylogenomics Laboratory, Ewha Womans University, Republic of Korea

Measurements. See Table 1.

Description. Female: Body cylindrical, length 366.3–483.1 μm, usually ventrally curved after fixation. Cuticle annulated; annuli 1.2–1.8 μm wide and 1.0–1.2 μm thick at mid-body. Lateral field occupying 16.0–21.1% of width of body at mid-body. Three incisures in lateral field, fading out at phasmid region. Head region continuous with neck. Six lips conoid, bearing 6+4 papillae. Transversal, oval-shaped amphidial apertures present. Three bifurcate labial probolae connected basally by tangential ridges, with seta-like or pointed termini. Stoma cephaloboid, length 1.6–2.0 times the lip region diameter. Cheilorhabdions oval- or bar-shaped. Small dorsal denticle sometimes present on metastom. Pharyngeal corpus cylindrical, 2.4–3.6 times isthmus length. Isthmus narrower than corpus, distinctly demarcated from metacorpus. Basal bulb oval-shaped with well developed valves; 1.2–1.4 times as long as its width.

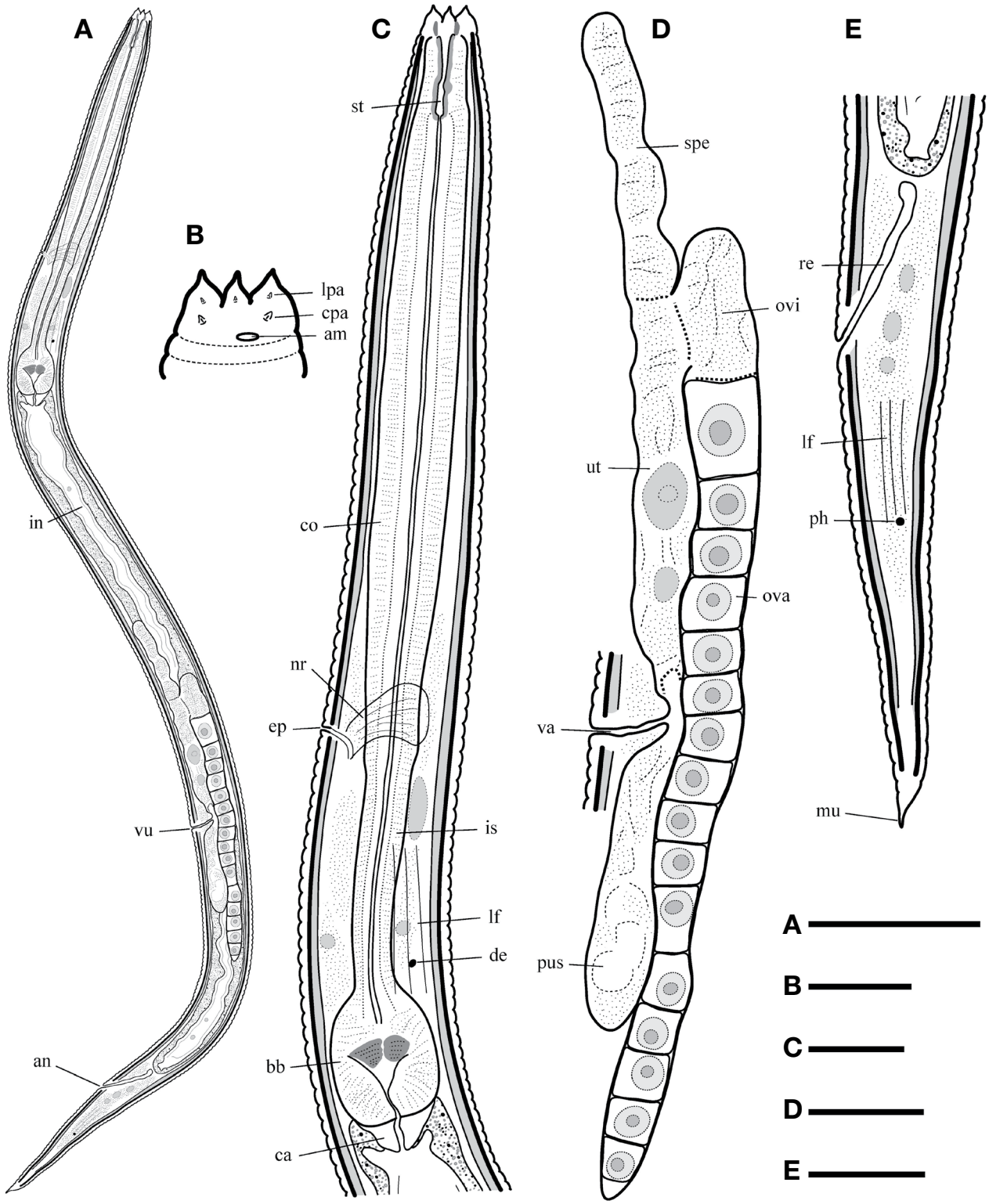

Fig. 3. *Eucephalobus hooperi* Marinari-Palmisano, 1967. A, Entire female; B, Head region; C, Female neck region; D, Female reproductive system; E, Female posterior region. am, amphid; an, anus; bb, basal bulb; ca, cardia; co, corpus; cpa, cephalic papilla; de, deirid; ep, excretory pore; in, intestine; is, isthmus; lf, lateral field; lpa, labial papilla; mu, mucro; nr, nerve ring; ova, ovary; ovi, oviduct; ph, phasmid; pus, post-uterine sac; re, rectum; spe, spermatheca; st, stoma; ut, uterus; va, vagina; vu, vulva. Scale bars: A=50 μm, B=5 μm, C–E=10 μm.

Cardia conoid, surrounded by intestinal tissue. Nerve ring located at posterior corpus or corpus-isthmus junction, at 59.1–67.9% of pharynx length. Excretory pore position at posterior corpus or corpus-isthmus junction, at 63.2–67.1% of pharynx length. Position of deirids in lateral field at isthmus or corpus-isthmus junction, at 68.7–75.5% of total neck length. Reproductive system monodelphic-prodelphic. Vulva lips not protruding. Vagina length 0.3–0.4 times the body diameter. Post-uterine sac 1.0–1.6 times the body width. Uterus 2.1–2.3 body diameters long. Spermatheca 0.9–2.0 times the body width. Oviduct short. Ovary straight, with a single row of oocytes. Rectum length 1.2–1.5 times the anal body diameter. Tail elongated-conoid, with pointed terminus. Spike-shaped mucro sometimes present. Phasmids located anterior to middle of tail, at 20.2–36.6% of tail length.
Male: Unknown.
Distribution. India, Italy, Kenya, Malaysia, South Korea, Spain.
Habitat. Soil sample from overgrown field and forest soil.
Remarks. Morphological characters of the specimen described in this study generally agree with other previous studies (Marinari-Palmisano, 1967; Boström, 1990, 1993; Abolafia and Peña-Santiago, 2002). However, the Korean population differs from the Italian populations in the ratio of corpus to isthmus length (2.4–3.6 vs 5–6) (Marinari-Palmisano, 1967). Compared to the Malaysian population, it differs in esophagus length relative to total body length (b = 2.9–3.5 vs. 3.6–3.8) and length of post-uterine sac relative to body width (1.0–1.6 vs. 0.7–0.8) (Boström, 1993). *Eucephalobus hooperi* is reported for the first time from South Korea.

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